

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
Benjamin Guy Davis, et al.	)	Examiner: Kosar, Andrew D.
Application No. 10/562,599	)	Art Unit: 1654
Filed: April 2, 2007	)	Confirmation No.: 6198
For: REAGENTS AND METHODS FOR	)	
THE FORMATION OF DISULFIDE	)	
BONDS AND THE	)	
GLYCOSYLATION OF PROTEINS	)	
	)	

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION OF BENJAMIN DAVIS UNDER 37 C.F.R. § 1.132

I, Benjamin G. Davis, hereby declare that:

1. I am a citizen of the United Kingdom of Great Britain and Northern Ireland, and am currently a resident of the city of Oxford in the country of England, which is part of United Kingdom of Great Britain and Northern Ireland.
2. I have been employed by The University of Oxford since 2001. I have many pending patent applications and have authored many papers in the area of carbohydrate chemistry. I am also one of the inventors of the above-identified patent application.
3. I graduated with a B.A. and D. Phil. degrees in chemistry from The University of Oxford. My undergraduate studies at The University of Oxford included the study of carbohydrate chemistry. Furthermore, I have been researching in the area of carbohydrate chemistry

since 1995. Hence, I am familiar with carbohydrate chemistry from both my education and my research.

4. I have reviewed B.G. Davis et al. et al. Tetrahedron: Asymmetry (2000) 11, pages 245-262, (hereinafter "the Davis publication"), L. Engman et al., Tetrahedron (1994) 50(9), pages 2929-2938 (hereinafter "the Engman publication"), H.S. Hsieh et al. Biochemistry (1975) 14(8), pages 1632-1636 (hereinafter "the Hsieh publication"), and U.S. Patent/Publication No. 5,759,823 by Wong, (hereinafter, "Wong"). I have also reviewed the above-identified patent application, and I have also reviewed what I understand to be the claims currently pending in the above-identified patent application. I understand that the Davis, Engman, Hsieh, and Wong publications have been cited against the above-identified patent application. I am one of the co-authors of the Davis publication.
5. Based on my review of the Davis, Engman, Hsieh, and Wong publications, I believe that one of ordinary skill in the art would not look to the Engman, Hsieh, and/or Wong publications in order to modify the reaction disclosed in the Davis publication.
  - a. I have reviewed the Engman and Hsieh publications and I do not believe that they would have led one of ordinary skill in the art familiar with the Davis publication to expect that the reaction of the presently claimed invention would be successful. I believe that one of ordinary skill in the art would know that the entire system must be considered when evaluating chemical reactions, including any side products and competing reactions that could affect the outcome. Furthermore, I believe that the nature of the reaction and its formation of either symmetrical or non-symmetrical (mixed) disulfides must be considered.
  - b. The reactions in the Engman and Hsieh publications relate to selenium-containing compounds, whereas those in the Davis publication relate to  $\text{SO}_2$ -containing

compounds. I believe that -Se- and -SO<sub>2</sub>- are not chemically equivalent, and *a priori*, there is no way of predicting whether these two moieties will have similar reactivities.

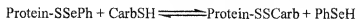
- c. I believe that the Hsieh publication as a whole relates to the formation of H<sub>2</sub>Se in biological systems and particularly in the presence of glutathione reductase. Thus, the reaction in equation 4 on the penultimate paragraph on page 1635 of the Hsieh publication notes that a tenfold increase in GSH concentration had little effect on H<sub>2</sub>Se production, whereas glutathione reductase was stimulatory at all concentrations. The reaction in equation 3 and equation 4 also uses a starting material, namely GSSeH, that is unrelated to the reaction claimed in the present application.
- d. One of ordinary skill in the art would realize that the reaction in the Hsieh publication forms a hazardous gas, namely H<sub>2</sub>Se. The formation of a gas is a different process with a different driving force than the reaction of the present invention. It is improper to draw any correlations between the reactions in the Hsieh publication and those of either the Davis publication or the present invention, since these reactions in either the Davis publication or the present invention do not conclude that a gas is being formed and do not use R-S-Se-H. Consequently, one of ordinary skill in the art cannot predict whether the reaction of the present invention will be successful on the basis of the Hsieh publication.
- e. Turning to the Engman publication, equation 3 of this document discloses a reaction in which two GSSePh molecules are in dynamic equilibrium with GSSG and PhSeSePh. The Engman publication notes that this reaction is catalyzed by

the presence of GSH, and proposes that the following equilibrium reaction occurs as part of equation 3:



The Examiner has identified this proposed step and asserted that one of ordinary skill in the art would realize that this has direct relevance to the reactions shown in the Davis publication. However, one of ordinary skill in the art would not necessarily assume that -Se- and -SO<sub>2</sub>- have the same reactivity.

- f. Moreover, I believe one of ordinary skill in the art would only view the reaction scheme shown in equation 3 in the Engman publication as a whole, and would not assume that the above isolated reaction, which is just one part of equation 3, is even favorable. For example, it may be that the actual driving force of the reaction shown in the Engman publication is the formation of PhSeSePh. Because an equivalent product cannot be formed in the reactions disclosed in the Davis publication, it would be clear to one of ordinary skill in the art that the reaction in equation 3 of the Engman publication is completely unrelated to the reactions disclosed in the Davis publication.
- g. Furthermore, the Engman publication clearly discloses that for each GSSG formed, the PhSeH that is obtained goes onto react with a further GSSePh to yield a GSH. If the total reaction of equation 3 in the Engman publication is applied to the reactions of the present invention, the expected outcome is as shown below:



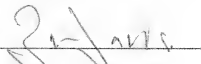
From the above, it is clear that the maximum expected yield for the reaction of the present invention based on the reactions shown in the Engman publication is only 50%, and protein thiol would be produced as an unrelated by-product.

Surprisingly, the Table in Example 19 shows that the actual observed protein conversion is over 95% and protein thiol is not observed. This would not have been expected, even if one of ordinary skill in the art had combined the teachings of the Engman and Davis publications. The reaction of the present invention therefore provides surprisingly high yields of mixed disulfides, which could not have been predicted, based on the teaching of Engman and the Davis publication.

- h. After reviewing the above-identified patent application, I understand that the above-identified patent application includes an embodiment of modifying a protein, peptide or amino acid comprising at least one selenenylsulfide group, by reacting this protein, peptide or amino acid with a carbohydrate compound comprising a thiol group.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Executed on 6/5/, 2011, at oxford, United Kingdom of Great Britain and Ireland.

By:   
Benjamin G. Davis